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Factors limiting heterotrophic bacterial production in the southern Pacific Ocean

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Abstract

The role of potential factors limiting bacterial growth was investigated along vertical and longitudinal gradients across the South Eastern Pacific Gyre. The effects of glucose, nitrate, ammonium and phosphate additions on heterotrophic bacterial production (using leucine technique) were studied in parallel in unfiltered seawater samples incubated under natural daily irradiance. Longitudinally, the enrichments realized on the subsurface showed three types of responses. From the Marquesas plateau (8° W to approx 125° W), bacteria were not bottom-up controlled, as confirmed by the huge potential of growth in non-enriched seawater (43±24 times in 24 h). Within the Gyre (125° W–95° W), nitrogen alone stimulated leucine incorporation rates by a factor of 5.6±3.6, but rapidly labile carbon (glucose) became a second limiting factor (enhancement factor 49±32 when the two elements were added). Finally from the border of the gyre to the Chilean upwelling (95° W–73° W), labile carbon was the only factor stimulating heterotrophic bacterial production. Interaction between phytoplankton and heterotrophic bacterial communities and the direct versus indirect effect of iron and macronutrients on bacterial production were also investigated in four selected sites: two sites on the vicinity of the Marquesas plateau, the centre of the gyre and the Eastern border of the gyre. Both phytoplankton and heterotrophic bacteria were limited by availability of nitrogen within the gyre, but not by iron. While iron limited phytoplankton at Marquesas plateau and at the eastern border of the gyre, heterotrophic bacteria were only limited by availability of labile DOC in those environments.

1 Introduction

Heterotrophic bacteria generally meet their energy and elemental needs from utilisation of organic matter, which includes essential elements like, C, N, P and Fe. However, in oligotrophic environments, elemental needs are sometimes not satisfied only by utilization of organic matter and heterotrophic bacteria can compete with phyto-

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plankton for mineral nutrients like N, P or Fe (Kirchman, 1994; Tortell et al., 1999; Thingstad, 2000). To examine factors limiting heterotrophic bacterial growth, seawater samples are generally amended with various components (organic molecules, macro nutrients, iron), alone or in combination. After 24–48 h, some bacterial parameters are examined, the main one primarily being bacterial production (either with thymidine or leucine technique). Different elements have been shown to stimulate bacterial production: phosphorus in the Atlantic Ocean (Sargasso Sea: Cotner et al., 1997, Gulf of Mexico: Pomeroy et al., 1995) and in the Mediterranean Sea (Eastern: Zohary and Robarts, 1998; Thingstad et al., 2005; Western: Van Wambeke et al., 2002), nitrogen in the South West Pacific Ocean (French Polynesia: Torréton et al., 2000), labile organic carbon in the Equatorial and Subarctic Pacific (Kirchman, 1990; Kirchman and Rich, 1997), iron in the Southern ocean (Pakulski et al., 1996, Tortell et al., 1996). From a punctual observation, it is difficult to generalize because within a given area, temporal and vertical variability have been shown (Sala et al., 2002; Van Wambeke et al., 2002). In addition, the simple point of view of "one single" resource limiting factor has evolved: i) co-limitation often occurs: carbon – iron (Tortell et al., 1999; Church et al., 2000; Kirchman et al., 2000), carbon – phosphorus (Sala et al., 2002, Van Wambeke et al., 2002), carbon – nitrogen (Torréton et al., 2000) ii) among heterotrophs, organic molecules might acts as nutrients for building biomass but also as energy resources; this observation is at the origin of the concept of energy limitation (Kirchman, 1990, Donachie et al., 2001) and iii) direct versus cascade effects: are bacteria directly stimulated, or do they benefit from a surplus phytoplankton production also affected by the relieving of one key nutrient (Palkuski et al., 1996; Church et al., 2000; Cochlan, 2001; Oliver et al., 2004; Obernosterer et al., 2007).

In the South Pacific Gyre, extreme isolation from terrestrial influence results in a permanent situation of hyperoligotrophy (Claustre et al., 2007[1]). Picoplanktonic organ-

[1]Claustre, H., Sciandra, A., and Vaulot, D.: Introduction to the special section: bio-optical and biogeochemical conditions in the South East Pacific in late 2004 – the BIOSOPE cruise, Biogeosciences Discuss., in preparation, 2007.

isms, both autotrophic and photoautotrophic, dominate community metabolism in this area (Grob et al., 2007). Bacterial production is very low in the centre of the gyre and relies upon autochthonous, photosynthetically derived organic matter as well as on changes in solar radiations as shown by the diel variability of bacterial production (Van Wambeke et al., 2007a, b[2]). Photo-autotrophic production is limited by nitrogen availability within the centre of the Gyre and is particularly adapted to the very low amounts of dissolved iron present (Bonnet et al., 2007). In contrast, iron limits phytoplanktonic production within the Marquesas plateau. The purpose of this study was to determine which factors limit bacterial growth in the South Pacific across longitudinal and vertical gradients.

2 Materials and methods

In order to identify the factors limiting heterotrophic bacterial production, two different sets of experiments were performed, one under trace metal clean condition (TMC), and the other under non trace metal clean conditions (non TMC).

TMC. These experiments were performed at four experimental sites. These stations represented different trophic regimes (Table 1): the mesotrophic area associated to the plume of the Marquesas Island (141.14° W, 8.19° S) (MAR), the adjacent medium nitrate, low chlorophyll waters (136.97° W, 9.04° S) (HNL), the hyperoligotrophic waters associated with the central part of the South Pacific gyre (114.02° W, 26.04° S) (GYR) and the oligotrophic eastern side of the gyre (91.39° W, 31.89° S) (EGY). The GYR site has been selected from ocean color images as having the lowest surface chlorophyll concentration in the world ocean. Details of these on-deck incubations are fully described in Bonnet et al. (2007). Briefly, seawater was collected at 30-m depth using a Teflon pump and dispensed into acid-washed (Suprapur Merck HCL) transparent

[2] Van Wambeke, F., Duhamel, S., Tedetti, M., and Claustre, H.: Heterotrophic bacterial production in the South East Pacific: daily variability, Biogeosciences Discuss., in preparation, 2007b.

polycarbonate bottles. Nutrients were added alone and in combination to final concentrations of 1 μM NH_4^+ (from ammoniac reagent) + 2 μM $NaNO_3$, 0.3 μM NaH_2PO_4, 2 nM $FeCl_3$, 10 μM C-glucose (Table 2). The bottles were incubated in an on-deck incubator with circulating surface seawater at appropriated irradiance (50% ambient light level). Three bottles for each treatment were randomly selected and sampled destructively at 24 h and 48 h. A large set of parameters were measured to follow nutrient concentrations, phytoplankton response (cytometric counts, variable fluorescence, chlorophyll, particulate primary production, Bonnet el al., 2007), bacterial abundance and heterotrophic bacterial production.

Non TMC. We also investigated more systematically factors influencing leucine incorporation rates using non TMC experiments, i.e. incubated water sampled from Niskin bottles and manipulated in a classical laboratory. Nutrients were added in order to obtain a final concentration of 1 μM NH_4Cl + 1 μM $NaNO_3$, 0.25 μM Na_2HPO_4, 10 μM C-glucose (Table 2). Transparent polycarbonate flasks of 60 ml were used and incubated on average 24 h in simulated in situ conditions (on deck incubator, neutral screens). For the longitudinal trend (141° W to 72° W, Fig. 1), seawater was sampled at one single depth varying from 5 to 30 m according the CTD profile. The depths chosen were always within the mixed layer, and corresponded to 50% incident light. We also investigated factors limiting BP along vertical profiles at sites GYR, EGY and UPW. The latter site corresponded to the upwelling area off the Chilean Coast (73.2° W, 33.5° S, Fig. 1). For those experiments, for each depth sampled, a series of five 60 ml polycarbonate flask (C, P, N, G, NPG) was incubated in a running seawater bath covered with a neutral screen corresponding to the incident light level. The deeper depth sampled was below the euphotic zone and was incubated in the dark in the incubator at in situ temperature. In situ light conditions were then correctly reproduced (excluding UV effects), whereas a slight difference could be obtained with temperature: up to 3°C difference at GYR (for the 185 m sample corresponding to 1% incident light: 19°C in situ, 22° during incubation), 2°C at EGY and 2.2° at UPW.

Bacterial abundance was determined by flow cytometry as described in Grob et

al. (2007). Bacterial production was determined using ^{3}H leucine technique coupled with the centrifuge method with full methodological description in Van Wambeke et al. (2007a). Briefly, seawater samples or sub-samples from incubated flasks were incubated in the dark for 1 to 2 hours after addition of 20 nM leucine. The terms "heterotrophic bacteria" and "heterotrophic bacterial production" are used in the whole text by simplicity but sensus stricto refers to heterotrophic prokaryotes and heterotrophic prokaryotic production, respectively. Indeed, prokaryotes include Bacteria and Archaea; and it has been shown that some organisms in both groups are able to incorporate leucine (Kirchman et al., 2007).

Nutrients were analyzed following standard colorimetric methods directly on board (Raimbault et al., 2007). Chlorophyll a (Chl *a*) concentrations given for the enrichment experiments were analyzed fluorimetrically as described in Bonnet et al. (2007) whereas concentrations were measured systematically at each station during the transect using HPLC method (Ras et al., 2007). Particulate primary production was measured on board in running seawater baths covered with a 50% neutral screen. Incubations periods lasted on average 5 h during morning time hours. Detailed methodology and assumption used to convert hourly to daily rates are fully described in Duhamel et al. (2007).

A significant stimulation was considered when the ANOVA comparison of distribution of the triplicates treatments gave $p<0.05$.

3 Results

3.1 Initial conditions prior to enrichment experiments

A large gradient of nutrient concentrations and chlorophyll a was observed along the transect (Table 1). Nitrate concentrations ranged from undetectable values to 3.6 μM (station STB 18), whereas soluble reactive phosphorus was always detectable and above 120 nM (Moutin et al., 2007). Chl *a* varied almost by 2 orders of magnitude

(0.02 mg m^{-3} in the centre of the gyre to 14.8 mg m^{-3} in the Chilean upwelling system). Leucine incorporation rates ranged from 10 to 164 pmol L^{-1} h^{-1}. Higher values of nutrients, Chla and leucine incorporation rates where obtained at the eastern part of the transect, within the Chilean upwelling (stations STB20, STA21, site UPW). Stations between STB6 and STB15 displayed lower Chla stocks and leucine incorporation rates (means ± sd 0.027±0.009 mg Chla m^{-3}, 11.9±1.2 pmol L^{-1} h^{-1}, respectively). Vertical profiles of dissolved iron indicated low (0.134±0.05 nM) and constant concentrations from the surface to 400 m throughout most of the transect (site MAR to station STA21), and increased notably from station STA21 to the Chilean coast, to reach 0.4 to 1.3 nM in surface waters (see also Blain et al., 2007).

3.2 TMC experiments

3.2.1 Heterotrophic versus autotrophic response

The extent of stimulation of phytoplankton and bacterial biomasses and productions following various amendments varied according to the station and the element tested. Roughly, phytoplankton was significantly stimulated by Fe addition at the MAR site, by Fe and N at the the HNL site, by N at the GYR site, and responded mainly to Fe+N(FeN) additions at the EGY site (Fig. 2, Table 4). Leucine incorporation rate was stimulated by Fe, but also N at the MAR site, glucose at the HNL site, N at the GYR site and by Fe+N and by glucose addition at the EGY site.

Besides the information concerning the limiting factor(s), we obtained the general following rules: stocks (chlorophyll *a*, bacterial numbers), when increasing, always increased less than the corresponding fluxes (primary production, leucine incorporation rates, Table 4). In addition, chlorophyll stocks increased after 48 h up to a factor of 6 (FeN at the HNL site), whereas bacterial abundances never increased more than a factor of 1.2 ("all" at the EGY site, GFe at the HNL site and "all" at the MAR site). Factors of increase of leucine specific activities were consequently very close to those of the fluxes, whereas primary production per unit of chlorophyll, when increasing,

increased, on average of two times less than photosynthesis rates (Table 4).

Out of these general trends, the intensity of the heterotrophic response differed from the intensity of autotrophic response. For instance, at the GYR site, both photosynthesis and heterotrophic production were stimulated after N additions (alone and combined: N, FeN, "all"), but leucine incorporation rates increased up to 8 times more than primary production (GYR all, after 24 h, ×16 and ×2, respectively, Table 4). On the opposite, in the treatments N and FeN at the HNL site and FeN in the treatments "all" at the EGY site, primary production increased on average 1.6 times more than leucine incorporation rates. It must be noticed that these latter cases corresponded to situations where phytoplankton and heterotrophic bacteria were not stimulated primarily by the same factor (i.e. N or Fe for phytoplankton and glucose for bacteria).

3.2.2 Iron Control on Heterotrophic Bacteria

At the MAR site, the most significant increase was obtained in the non-amended control (×20 in 24 h), whereas Fe alone, macronutrients (N, P Si) or a combination of all the nutrients (Fe, N, P, Si) lead to similar response (1.8 to 2.2 times higher than the control at the same time). There was no other additional effect after 24 h. At the HNL site, only the glucose and glucose + Fe additions resulted in a significant increase of leucine incorporation rates (×12 and ×22), visible 24 h after enrichment. At the GYR site, Fe + N (FeN), and a combination of all the nutrients Fe + N + P (All) resulted in a significantly higher leucine incorporation rates after 24 h (×11, ×16, respectively) and 48h (×17, ×23, respectively). Although the leucine incorporation rate was also enhanced after N addition alone, the difference with the control was only significant after 48 h (×9). Glucose (alone or in combination with Fe) did not result in a significant increase of leucine incorporation rate, even after 48h. At the EGY site, however, only glucose additions after 48 h enrichment had significant effect compared to the control (×3.5 in G and ×2.3 in GFe, Table 4, Fig. 2).

The results obtained in the framework of these trace metal clean experiments allowed us to draw some conclusions: i) iron was never a single factor limiting bacterial

production, and consequently ii) effects of N, P and glucose additions could be studied more systematically along the transect by using "non TMC" technique, ii) 24 h incubations should be long enough to detect some stimulation, if occurring.

3.3 Non TMC experiments

3.3.1 Longitudinal variability of nutrient control on heterotrophic bacteria

Additions of elements were realized in triplicate conditions only at the station STB18. Results from this experiment were used to define a threshold for the significant effect of any enrichment for other experiments which were not replicated (Fig. 3). Variability within triplicates ranged from 5 to 39%, mean 22%. ANOVA test in this bioassay resulted in a significant effect for G ($p<0.005$) and NPG ($p<0.001$) compared to the control. Considering the mean factor of enrichment reached with the different combinations at this station (0.8, 1.3, 2.6 and 4.1 for P, N, G, NPG, respectively), we assumed that a factor 2 of increase (compared to the non-amended control) should be a minimum threshold to confirm a positive effect when experiments were not performed in triplicate.

Along the horizontal transect in the mixed layer, P alone stimulated leucine incorporation rates only in 1 case over 23 tested (station STB4, ×2.2 higher than the control). However, in that bioassay the addition of N (×3.4) and G alone (×2.6) also resulted in an increase of leucine incorporation rates. Effects of N, glucose and NPG additions on leucine incorporation rates showed three groups of responses (Fig. 4). In the western part, from the MAR site to station STB5, the leucine incorporation rate was greatly stimulated simply by confinement during 24 h in a polycarbonate bottle. On average, the leucine incorporation rates increased in the non-amended control by a factor of 43±20 (Fig. 4a). This is in agreement with the TMC experiments conducted at the MAR site (Fig. 2). The median values of stimulation factors were ×1.4, ×1.1 and ×2.6 for glucose, N and NPG addition, respectively (Fig. 4b). The NPG stimulation factor was higher than the threshold "2" on 3 occasions over the 6 stations tested, and N and

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G factor only once. In the second group of stations (STB6 to STB15, including the GYR site), the leucine incorporation rates increased in a lower ratio (×1.1 to ×5.3) in the non-amended control. The median values of stimulation factors for glucose, N and NPG additions were ×1.4, ×4.2 and ×37.2, respectively. The N stimulation factor was higher than the threshold of "2" on 8 over 9 stations tested, and G factor on 2 over 9, but in these 2 cases, N alone also stimulated leucine incorporation rates. For the last group of stations (EGY to UPW2), the leucine incorporation rates also increased slowly in the non-amended control (×2.1 to 2.7 in 24 h between station EGY and STA21, but ×4.1 and ×3.5 at stations UPW1 and UPW2). The median values of stimulation factors for glucose, N and NPG additions were ×1.7, ×1.1 and ×3.4, respectively (Fig. 4b). The increase after glucose addition was higher than the threshold "2" in 2 of the 8 stations tested, and the increase after NPG addition was higher than the threshold "2" in 7 of the 8 stations tested.

3.3.2 Vertical variability of nutrient control on heterotrophic bacteria

There were also varying stimulation factors of leucine incorporation rates along vertical profiles. At the GYR site, leucine incorporation rates increased after N addition at all depths tested, even if nitrate was significantly detected below 150 m (0.15 μM at 185 m, 3.6 μM at 245 m, Fig. 5). Glucose, however, has an higher effect than N at 185 m (×83 versus ×4) and 245 m (×495 versus ×190, respectively). At the EGY site, the stimulation factor of leucine incorporation rates after 24 h addition of glucose progressively increased between 80 m and 250 m (×4 up to ×105). Stimulation by N alone was non existent or low (×2.2 at 40 m, ×2.6 at 250 m), although nitrogen was still undetectable below 40 m. Stimulation by NPG also increased from the surface layer (×1.1–×2.9 below 30 m) to 250 m (×49). At the UPW site, only effects of G and NPG were visible, being more or less constant and with poor increases (factors <4) below 10 m. At 5 m depth, none of the nutrients (alone or in combination) stimulated significantly leucine incorporation rates.

4 Discussion

4.1 Abundance or production for tracking limitation?

Because both abundance and leucine incorporation rates have been followed, one question is arising: What is the best indicator for tracking factors limiting heterotrophic bacterial growth? The increase in leucine incorporation rates, when present, is either due to stimulation of a greater percentage of active population, or the stimulation of the specific growth rate of individual cells, or some combination of these two processes. Generally, bacterial abundance indeed responds less than production to enrichments (Kirchman, 1990; Pomeroy et al., 1995; Carlson and Ducklow, 1996; Graneli et al., 2004). Bacterial abundances indeed showed a response up to ×2 after additions, whereas leucine incorporation rates increased up to 23 times (Table 4), confirming this previous results. Unbalanced growth is often the explanation for the strong changes generally observed with the thymidine or the leucine technique, compared to that of abundances (Carlson and Ducklow, 1996). Biovolumes have not been quantified systematically in this study, but preliminary observation of slides after fluorescent in situ hybridization (results not shown) revealed enhancements of average biovolumes. These preliminary results also suggest a shift in some bacterial populations, which is also sometimes observed after long-term periods of confinement and enrichments (Pinhassi et al., 2006). Rapid growth of gamma-proteobacteria after FeN and "all" additions (Van Wambeke, unpub. results) was effectively observed at the GYR site. Finally the number of heterotrophic flagellates also increased, suggesting that bacterial predation was also enhanced after some stimulation of heterotrophic bacteria (GYR site, N enrichments, Table 4), and consequently regulated bacterial abundances. In conclusion, the leucine incorporation rates were thus better indicators than abundances for tracking factors limiting bacterial growth. Nevertheless, responses could have been different in terms of intensity or delay with other tracers of heterotrophic activity (Carlson and Ducklow, 1996; Donachie et al., 2001).

4.2 Direct versus indirect limitation

There has been much debate about direct (suppression of a real limiting factor) or indirect (stimulation of phytoplankton by the added component which induces a surplus of DOM production fuelling heterotrophic bacteria) effects of iron and other macronutrients limitation on heterotrophic bacteria (Table 4).

The timing and magnitude of responses of phytoplanktonic parameters (primary production, chlorophyll biomass) compared to that of heterotrophic bacteria in the experiment performed under TMC have helped to argue for possible direct or indirect effect of nutrient additions. If iron alone induced a positive stimulation of leucine activity within the Marquesas plateau (MAR site), addition of other nutrients N+P+Si ("all") without Fe stimulated leucine activity at the same level (factor 2 compared to the control at the same time, Fig. 2). In addition, chlorophyll biomass increased compared to the control, by a factor equivalent to that of leucine activity in 24 h, and even more after 48 h (factor 3, Table 4). It is thus probable that the effect of Fe on leucine activity was indirect. The major effect obtained at the MAR site is however obtained in the non-amended control (×20 at the MAR site in the "trace metal clean" conditions, Fig. 2), compared to the response of phytoplankton in this control (Chl*a* did not increase significantly in the control, Table 4). It is possible that sufficient amounts of labile DOC were present, allowing a rapid bacterial growth without any enrichment, which implies that heterotrophic bacteria were not limited at all after a 24 h confinement at the MAR site. In contrast, phytoplankton at the MAR site was under a high Fe stress, which was clearly visible from analysis of photochemical efficiency of photosystem II (Bonnet et al., 2007). The capacity of bacteria to grow on bulk DOC was also seen in the vicinity of MAR site, up to 125° W, as a strong growth in the non-amended controls was observed up to station STB5 (Fig. 4a). Interpretation of growth in a non-amended control is difficult because the use of batch experiments last for a few days. This implies to take into account bottle effect: underestimation of the levels of the trophic web, and possible destruction of fragile cells during handling that fuels labile organic resources for heterotrophic bacteria.

However, all the other experiments have been performed in the same conditions, and the non-amended control did not increase that much. Possible other explanations are the change in the top-down control of bacteria or non steady state of bacterial growth at the time of the sampling at these stations. Day-to-day changes in in situ primary production, as well as in situ specific leucine incorporation rates have been observed during occupation of the MAR site (Van Wambeke et al., 2007a) and argue for this second hypothesis.

At the GYR site, the addition of iron did not result in any significant increase of photosynthesis rates (Table 4). Although dissolved iron was low and constant along most of the transect (~0.1 nM in surface), it was shown that phytoplankton was acclimated to iron deprivation in the centre of the gyre (Bonnet el al., 2007). N addition alone (nitrate + ammonium) or in combinations (FeN, "all") have all stimulated chlorophyll and primary production (24 and 48 h) and specific primary production (48 h). Leucine incorporation rates increased also after the addition of N, as soon as 24 h, although there was no significant stimulation by glucose, alone or in combination with iron. This suggests that, in the gyre, stimulation of bacterial production by nitrate + ammonium addition is direct. However, if nitrogen limitation is a direct effect, and considering responses of BP after other combination of enrichments, co-limitation occurred rapidly. At the GYR site, N (nitrates + ammonium), FeN (Fe + N) and all (Fe + N+ P) treatments stimulated exactly to the same degree phytoplankton properties (chlorophyll, primary production), whereas the leucine incorporation rate was stimulated more progressively in these three treatments. This suggests that a labile organic carbon source could also limit BP after relieving the N limitation. This result is confirmed by the "non TMC" experiments, which show that the stimulation was greater after the addition of N + P + glucose than after the addition of N alone (nitrates + ammonium) in most of the waters tested within the center of the gyre (Fig. 3, see STB6 and Fig. 4b, see STB6 to STB15). This thus suggest that in the area between 122° W and 95° W, labile carbon rapidly becomes a co-limiting factor after N for bacterial production. Within the deep chlorophyll maximum (DCM), however, glucose is the first limiting nutrient, not N (Fig. 5). A similar

switch from a mineral nutrient within surface layer to organic C limitation within DCM has been evidenced in the Mediterranean Sea (Sala et al., 2002; Van Wambeke et al., 2002).

The rapidity of the response of heterotrophic versus autotrophic response after relieving the N limiting nutrient in the South Pacific gyre could have consequences on the metabolic balance of this environment. Recently, it has been shown that 5–10% of deep sea water mixed with water from the nutrient-limited mixed layer of the North Pacific Gyre provided a set of nutrient enrichment sufficient to significantly enhance the net community production after 5 days (Mc Andrew et al., 2007). Thus, the question arises here if, in our nitrogen enrichment experiments made at the GYR site, autotrophic process were also notably favoured in regard to respiration. Oxygen budgets were not measured in our experiments, but as our stimulation factors were much greater with leucine incorporation rates than particulate primary production, our results would suggest the opposite (i.e. enrichment would favour heterotrophy at the GYR site). Nevertheless, the leucine incorporation rate is not respiration, and possible changes in leucine conversion factors, as well as in bacterial growth efficiencies with time could not be ruled out. Indeed, the relieving of a factor limiting heterotrophic bacteria enhances bacterial growth efficiency (Carlson and Ducklow, 1996). Also, our experiments lasted only 48 h, whereas significant chlorophyll biomass and net community production occurred only after 4–5 days in the Mc Andrew et al. (2007) experiments. Finally, the rates at which autotrophic and heterotrophic cells developed in our experiment might have been influenced by enzymatic properties of uptake systems for ammonium and nitrate which differs inside these two groups, heterotrophic bacteria being favoured by ammonium addition, whereas only nitrate were provided by deep sea water enrichments in Mc Andrew et al. (2007) approach.

4.3 Carbon versus energy limitation of heterotrophic bacteria

Because the organic molecule tested is also often an energy-rich molecule easily entering catabolic pathways (e.g. glucose), one could wonder if this carbon source is

tracking carbon resource limitation or energy limitation (Table 4). Glucose was the only organic molecule tested in our study, and we examined its potential use by comparison of time-integrated bacterial carbon demand during the course of bioassays, and compared it to the amount (10 μM C) of glucose added. Integrated BP during 24 h was calculated based on values of leucine incorporation rates measured before and after 24 h of glucose addition, assuming an exponential rate between these 2 measurements, and a leucine conversion factor of 1.5 kg per mole leucine incorporated (Van Wambeke et al., 2007a). Then, bacterial carbon demand was calculated assuming that glucose is taken-up by bacteria with a 20% efficiency. This comparison suggests that all the glucose added can be used within 24 h in the NPG combinations, and some use of the in situ DOC is also possible. Free glucose (not combined) is extremely rare in this area (Sempéré et al., 2007[3]), suggesting that this molecule plays an important role in energy restoration once added. DOC is probably highly refractory, due to strong UV radiation effects. Indeed, it has been shown that growth of heterotrophic bacteria at the GYR site after a one day UV exposure of DOM is partly inhibited (Sempéré et al., 2007[3]). It is probable that the limitation in N and energy prevents the synthesis of enzymes able to degrade the bulk accumulated DOC present in this area. Indeed, in addition to changes in populations (Pinhassi et al., 2006), induction of some genes after the relieving of some limiting nutrients has been observed (Arietta et al., 2004). Bacteria were described as energy limited in the South Pacific (Kirchman, 1990), and in the Eastern North Pacific (Cherrier et al., 1996), but resource limited in the Sargasso Sea (Carlson and Ducklow, 1996). In addition, conflicting interpretation are sometimes given to the same observation, for instance when comparing enhancement of bacterial activity after amino acids addition or with glucose + ammonium addition (Kirchman, 1990; Carlson and Ducklow, 1996; Donachie et al., 2001). It is probably impossible to be categorical on the question of energy-only versus resource-only limitation in nature

[3]Sempéré, S., Tedetti, M., Charrière, B., Panagiotopoulos, C., and Van Wambeke, F.: Molecular distribution and bacterial availability of dissolved sugars in the south East Pacific, Biogeosciences Discuss., in preparation, 2007.

and varying conclusions given probably rely on the quality of organic matter present and the parameter tracking heterotrophic activity.

In the centre of the South Pacific Gyre, nitrogen was a common primarily factor limiting phytoplankton and bacteria. Competitive advantages for N between bacteria and phytoplankton might thus exist and vary with irradiance levels, which had not been considered in this study. Indeed, it has also been suggested that the factors limiting bacteria could change at a daily scale (Shiah, 1999; Kuipers et al., 2000). DOC produced by phytoplankton release could be a major source of carbon and energy for bacteria. The huge needs of nitrogen and energy source for heterotrophic bacteria, as well as their strong plasticity to grow again once limitation is relieved, are probably the key factors explaining the strong coupling between phytoplankton and bacteria as seen on diel cycles (Van Wambeke et al., 2007b[2]).

5 Conclusions

A large set of enrichment experiments was performed here along a 8000 km transect, sometimes along vertical profiles, allowing a broad generalization of the observed trends over a large spatial scale of the South Pacific Ocean in an austral summer situation. Our enrichment experiments have shown that iron was never the single nutrient limiting bacterial production. In the vicinity of the Marquesas Islands, our results showed stimulation by iron alone and nitrogen alone suggesting that stimulation by iron was an indirect effect. Nitrogen was the first factor limiting heterotrophic bacterioplankton within the surface layers in the centre of the south Pacific Gyre, rapidly followed by a co-limitation with labile carbon. However, at the bottom of the euphotic zone within the deep chlorophyll maximum there is a switch to carbon limitation. Overall the surface layers around the borders of the South Pacific Gyre (Marquesas plateau, upwelling off Chile), labile carbon was the primarily factor limiting heterotrophic bacterial production.

Because the weak horizontal advection in the centre of the South Pacific Gyre and because intense seasonal convective mixing is excluded (Raimbault et al., 2007), this

suggests that this situation of nitrogen limitation could be permanent throughout the entire year. However, nitrogen was a common primarily factor limiting both phytoplankton and bacteria, which probably had consequences on their relationships at short time scales. The heterotrophic bacterioplankton of the South Pacific Gyre lives in a very dynamic situation which is difficult to determine adequately, and current methods and tools for detecting limiting factors on short incubation time are urgently needed.

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Table 1. Initial conditions of "trace metal clean" experiments. BN: bacterial abundances. PP: primary production.

	NO_3 μM	NH_4 μM	PO_4 μM	$SiOH_4$ μM	Fe nM	Chla mg m^{-3}	PP mgC m^3 d^{-1}	BN $\times 10^5$ ml^{-1}	leu inc rate pmol $L^{-1}h^{-1}$
MAR	1.97	0.32	0.37	0.97	0.13	0.18	nd	9.6	26
HNL	1.82	0.04	0.31	0.91	0.14	0.11	9.1	8.9	27
GYR	ld	ld	0.11	0.55	0.1	0.03	1.8	4.1	18
EGY	0.04	0.008	0.17	1.02	0.1	0.07	6.5	7.5	15

Table 2. Summary of enrichment conditions. Fe: iron, NH_4: ammonium, NO_3: nitrates, Si: orthosilicic acid, PO_4: phosphates, GLU: glucose.
* "trace metal clean" conditions of manipulation,
** Si addition resulted in a slight contamination in Fe of about 0.1 nM,
*** non "trace metal clean" conditions of manipulation

experiment	code	Fe	NH4	NO3	Si	PO4	C-GLU	Incubation sampling
MAR*	C							24–48 h
	Fe	2 nM						in situ
	N		1 μM	2 μM	2 μM**	0.3 μM		simulated
	all	2 nM		2 μM	2 μM**	0.3 μM		50% light screen
HNL*	C							24–48 h
	Fe	2 nM						in situ
	N		1 μM	2 μM	2 μM**	0.3 μM		simulated
	FeN	2 nM		2 μM	2 μM**	0.3 μM		50% light screen
	G						10 μM C	
	GF	2 nM					10 μM C	
GYR*	C							24–48 h
EGY*	Fe	2 nM						in situ
	N		1 μM	2 μM				simulated
	FeN	2 nM	1 μM	2 μM				50% light screen
	all	2 nM	1 μM	2 μM		0.3 μM		
	G						10 μM C	
	GF	2 nM					10 μM C	
routine	C							24 h
bioassays***	P					0.25 μM		in situ
	N		1 μM	1 μM				simulated 50%
	G						10 μM	light screen
	NPG		1 μM	1 μM		0.25 μM	10 μM	

Table 3. Initial conditions prevailing in sea water samples used for routine bioassays. SST: sea surface temperature (used in running sea water baths during incubations), Chla: Chlorophyll *a*, Leu inc rate: incorporation rates of leucine into proteins, ld: below detection limits. * refers to data acquired on another CTD cast sampled in an interval of few hours on the same site.

station	depth m	Longitude °W	Latitude °S	date	SST °C	NO_3 μM	NH_4 μM	PO4 nM	Chla mg m^{-3}	Leu inc rate pmol $L^{-1}h^{-1}$
MAR	5	141°14	08°19	29-Oct		1.68*	0.33*		0.168	50.9
STB1	10	134°05	11°44	3-Nov	27.8	0.70 (5m)	0.03	313	0.112	30.9
STB2	15	132°06	13°33	4-Nov	27.4	ld	0.01	208	0.088	29
STB3	15	129°55	15°32	5-Nov	27.1	0.046	0.004	193	0.055	21.4
STB4	15	127°58	17°14	6-Nov	26.5	ld	ld	213	0.050	21
STB5	20	125°33	18°44	7-Nov	25.7	ld	ld	163	0.038	15.9
STB6	25	122°53	20°27	8-Nov	24.5	ld	0.008	178	0.018	11.9
STB7	20	120°22	22°03	9-Nov	24.3	0.046	ld	143	0.022	13.4
STB8	30	117°53	23°33	10-Nov	23.4	ld	ld	128	0.026	12.4
GYR	30	114°00	25°58	12-Nov	22.1	ld	0.02*	128	0.028	10.8
STB11	30	107°17	27°46	20-Nov	21.3	ld	ld*	123	0.032	12.4
STB12	30	104°18	28°32	21-Nov	21.2	ld	0.001*	133	0.022	11.2
STB13	25	101°28	29°13	22-Nov	20	ld	0.003*	123	0.023	10.6
STB14	20	98°23	30°02	23-Nov	19.8	0.048	ld*	138	0.027	10.5
STB15	15	95°25	30°47	24-Nov	18.7	ld	0.015*	153	0.048	13.9
EGY	15	91°27	31°49	26-Nov	18.1	0.006*	0.006*	178	0.074	17
STB17	15	86°47	32°23	1-Dec	17.3	2.65*	0.116*	313*	0.116	20.3
STB18	15	84°04	32°40	2-Dec	17.4	3.64	0.119*	388	0.147*	16.6
STB19	15	81°12	33°01	3-Dec	17.2	2.76	0.132*	373	0.066	17.5
STB20	5	78°07	33°21	4-Dec	17.6	0.095		268	0.274	51.5
STA21	5	75°49	33°36	5-Dec	16.8	0.071		358	0.218	54.6
UPW1	5	73°22	33°59	6-Dec	15.9				1.481*	145.4
UPW2	5	73°21	33°55	7-Dec		0.289		508	1.394	163.7

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Table 4. Table 4. Summary of the factors of increase obtained after 24 and 48h incubations in "trace metal clean" experiments. Results are expressed as the ratio of the value obtained divided by the value obtained in the non-amended control at the same time. For the lines corresponding to non-amended controls (C), the italic, underlined values indicate the factor of increase relative to the control at time zero. Enrichment codes correspond to Table 1. The parameters and their units before comparison are: chlorophyll a (Chl a, mg m^{-3}), bacterial abundance (BN, cells ml^{-1}), heterotrophic nanoflagellate abundances (HNAN, cells ml^{-1}), ^{14}C-primary production (PP, mg C m^{-3} d^{-1}), leucine incorporation rates (Leu pmol L^{-1} h^{-1}), primary production per unit Chla (spec PP, mgC mg $Chla^{-1}$ d^{-1}) and leucine incorporation rate per cell (spec Leu, $\times 10^{-21}$ mol $cell^{-1}$ h^{-1}). Empty places: parameter not sampled. * Indicates a response significantly different at $p<0.05$. ** Indicates a response significantly different at $p<0.0001$ [a] For HNAN statistical comparison was unavailable (only one sample, no triplicates).

		24 h							48 h						
		Chl A	BN	HNAN[a]	PP	Leu	spec PP	spec Leu	Chl A	BN	HNAN[a]	PP	Leu	spec PP	spec Leu
MAR	C	*1.5*	*1.1*			*20.1*		*17.6*	*2.0*				*17.4*		
	Fe	1.6	1.1			1.8*		1.7*	3.0*				2.1*		
	N	1.7	1.1			2.1*		1.8*	1.7				1.5		
	all	2.2*	1.2			2.2*		2.0*	8.7**				2.3*		
HNL	C	*1.4**	*1.1*	*1.2*	*1.1*	*4.2*	*0.8*	*3.8*	*1.3**	*1.2*	*1.3*	*0.8*	*2.6*	*0.6**	*2.2*
	Fe	1.4*	1.0	1.1	2.8*	3.1	2.0**	3.3	3.6**	0.9	1.2	5.5**	4.6	1.5*	5.0
	N	1.9*	1.0	1.8	3.9**	2.3	2.1**	2.2	4.5**	1.0	2.1	5.7**	2.7	1.1	2.7
	FeN	1.9*	0.9	1.7	4.3**	2.9	2.3**	3.1	6.1**	1.0	2.0	9.5**	8.7	1.4	9.1
	G		1.1*	1.4		11.9*		10.5*		1.1	1.4		6.3		5.5
	GF		1.2*	1.2		21.7*		18.8*		1.1	1.4		19.1*		16.3*
GYR	C	*1.1*	*0.9*	*1.1*	*0.4***	*0.8*	*0.4***	*0.9*	*0.7*		*1.1*	*0.5**	*1.0*	*0.7**	
	Fe	1.5*	1.0	1.0	1.1	1.4	0.6*	1.4	1.0		1.0	0.7	0.5	0.7	
	N	2.2**	1.0	1.1	2.1*	4.6	0.9	4.7*	3.1**		1.4	4.2**	8.9*	1.4*	
	FeN	2.3**	1.0	1.1	2.4**	11.0*	1.1	11.0*	4.0**		1.6	5.4**	16.9*	1.4*	
	all	2.1**	1.0	2.0	2.0*	16.0*	0.9	15.9*	3.3**		1.8	4.5**	23.4*	1.4*	
	G		1.0	1.1		3.0		3.1			1.4		3.5		
	GF		1.0	1.0		3.4		3.5			1.5		4.9		
EGY	C	*1.9**	*1.1*	*1.1*	*0.7***	*3.1*	*0.4***	*2.2*	*1.8**	*1.1*	*1.1*	*0.8**	*3.5*	*0.4***	*2.5*
	Fe	0.9	1.0	1.2	1.0	1.4	1.1	1.4	1.4	1.1	1.6	1.3*	1.5	1.3*	1.3
	N	1.2	1.0	1.3	1.1	1.3	0.9	1.3	1.6*	1.0	1.9	1.9**	1.2	1.2*	1.2
	FeN	1.2*	1.0	1.2	1.5**	1.1	1.0	1.1	2.6**	1.0	2.0	3.8**	1.9	1.5**	1.8
	all	1.3	1.0	1.6	1.8**	0.9	1.4*	1.0	3.0**	1.2	2.4	4.5**	2.0	1.5**	1.7
	G		1.0	1.2		1.4		1.4		1.0	1.4		3.5*		3.4*
	GF		1.0	1.1		1.8		1.7		1.0	1.7		2.3*		2.4*

Table 5. Review of some factors enhancing some bacterial variables among different oceanic environments (Leu, Tdr: production based on Leucine and thymidine technique, BN: bacterial numbers, BS: bacterial size, EEA: ectoenzymatic activities, O2: respiration, BGE: bacterial growth efficiencies, DIV: diversity). DCM deep chlorophyll maximum, PE-DOM : filtrate of heat killed plankton extract (>335 μm), Me+Vit: trace metals + vitamins, glu: glucose, DFAA: dissolved free amino acids. Co refers to colimitation. *in situ fertilization experiments. When carbon is limiting, and when the information was available, the terms in italic (*resource, not clear and energy*) refer to the authors' conclusions about energy versus resource limitation.

area	date	parameter followed	nutrients tested	limitation by	effect direct vs cascade	
Gulf of Mexico to Mississippi. river plume	Jan June 1993	Leu, BN, O2	NH4, PO4, Me,+Vit, glu	P		Pomeroy et al 1995
Sargasso Sea off Bermuda	July Oct 92, March July 93, Jan 94	Leu, Tdr, BN, BS	NH4, PO4, glu, DFAA, algal lysate	C *(resource)*		Carlson and Ducklow 1996
Med Sea, Western, Ionian, Levantine	June & Sep 99	Leu	NO3, PO4, glu	co C-P, P		Van Wambeke et al. (2002)
Med Sea, Catalano-Balearic Basin, surface, DCM	June 95, June Sep 96	Leu, BN	NO3, PO4, glu	P, co C-P		Sala et al. (2002)
				C, N		
Med Sea, Ionian, Cretan, Levantine Basins	Jan Feb 1995	Tdr, BN	PO4, NH4,Fe, EDTA	P		Zohary and Robarts (1998)
Med Sea, Levantine (Cypprus Gyre)		Leu, BN	PO4 *	P	direct	Thingstad et al. (2005)
Subarctic Pacific, gulf of Alaska	Sep 1987, May Aug 1988	Tdr, Leu, BN	NH4, DFAA, glu, protein, alkylamines	C *(energy)*		Kirchman (1990)
Subtropical north Pacific, St ALOHA	Dec 96-April 98	glucose uptake, EEA	NH4, NO3, leu, his, glu	co N-C *(not clear)*		Donachie et al. (2001)
Eastern North Pacific, California current	June Oct 1992	O2, BN, BGE	PE-DOM,glu, NH4, urea, PO4, dfaa	C *(energy)*		Cherrier et al. (1996)
Eastern North Pacific, California current	June 96, June 97	Leu, BN	Fe		not clear	Hutchins et al. (1998)
Eastern equatorial Pacific, IRONEX II	May 1995	Leu, BN	Fe *	Fe	not clear	Cochlan 2001
South East Pacific, Tuamotu atolls	Nov 95, March 96	Tdr	NH4, PO4, glu	N, C, P, co C-N		Torréton et al. (2000)
Ocean around atolls				C	direct	
South eastern Pacific Gyre (this study)	Nov–Dec 2005	Leu, BN	Fe, NH4+NO3, PO4, glu	N	direct	This study
Antarctic, Gerlashe Strait	Oct 1995		Fe	Fe	direct	Palkulski et al. (1996)
Southern ocean, Atlantic sector	austral summer 97/98	Leu, BN	NH3 PO4 glu	C		Tortell et al. (1996)
Southern ocean, Atlantic sector, EISENEX	Nov 2000	Leu, Tdr, BN, EEA, DIV	Fe*	Fe	not clear	Arrieta et al. (2004)

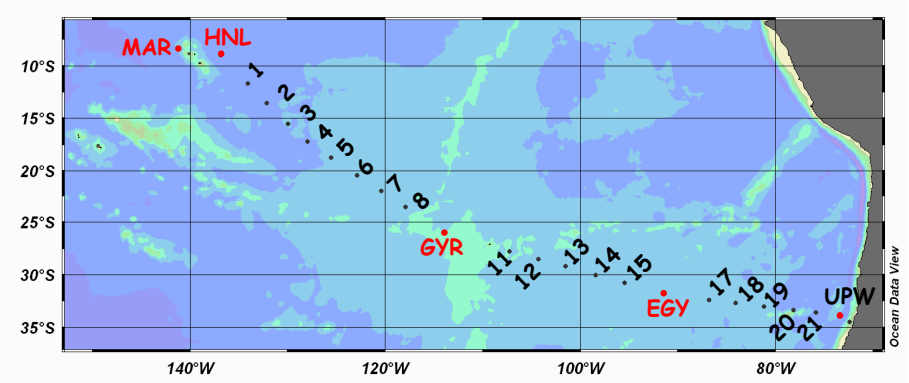

Fig. 1. Transect of the BIOSOPE cruise from the Marquesas Islands to Chile. In red, stations where "trace metal clean" enrichment experiments were processed, in black, stations used for "non metal clean" bioassays. Numbers indicates short-term stations, for which only numbers have been indicated to simplify presentation, not the complete code as in Table 3. For instance 1 is STB1 and 21 is STA21.

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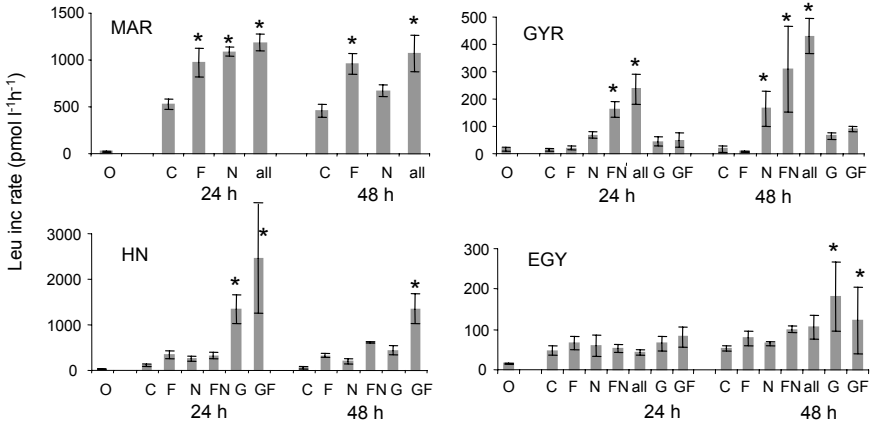

Fig. 2. Evolution of leucine incorporation rates on bioassay experiments made under "trace metal clan" conditions. Means ± sd of triplicate bottles sampled after 24 and 48 h of incubations. For MAR and HNL enrichments were O: initial conditions, C: control nonenriched, F:+iron, N:+ nitrates+ammonium+phosphates+silicates, all: nitrates+phosphates+silicates+iron, G:+glucose, GF:glucose + iron. For GYR and EGYR N was only nitrate+ammonium, FN was nitrate+ammonium+iron and all nitrates+ammonium+phosphates +iron. *:response significantly different from the control at the same time, ANOVA test, $p<0.05$.

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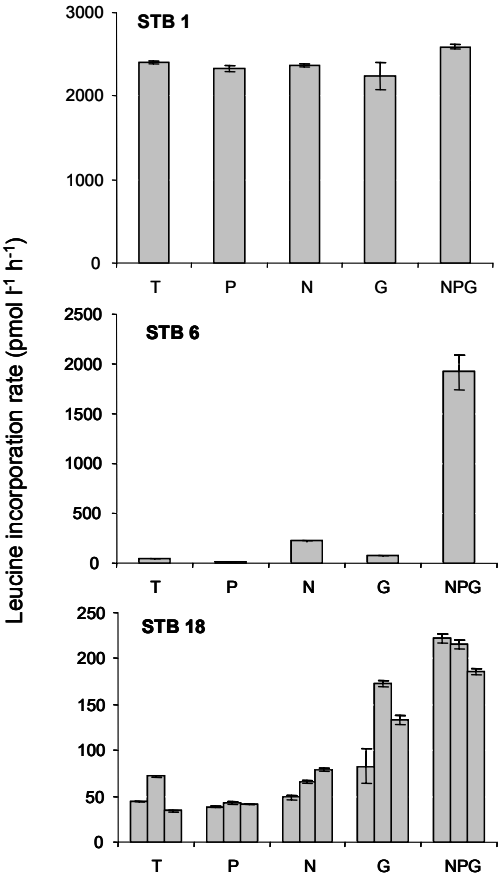

Fig. 3. Typical results obtained from "non metal clean" bioassays. C: control, P: + phosphates, N: + nitrites + nitrates, G: + glucose, NPG: all 4 elements. Error bar represents variability within duplicate leucine measurements in a single flask. At station STB18, triplicate flasks were incubated. Station STB1: no stimulation, STB6: slight stimulation with N, big stimulation with NPG, STB18: stimulation with glucose.

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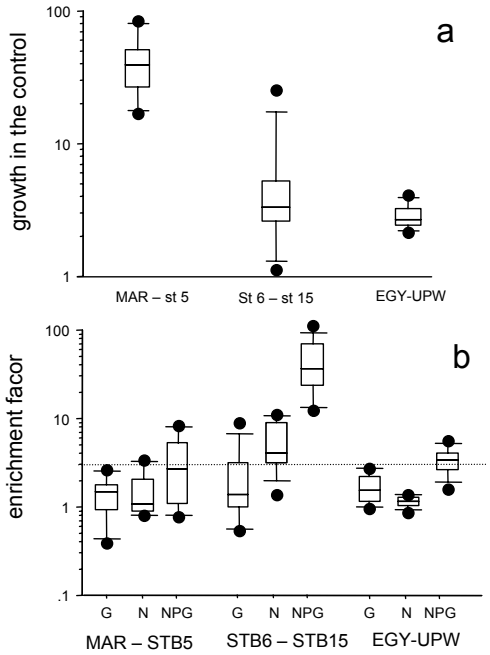

Fig. 4. Mean results on bioassays realized in sub surface waters, varying from 5 m (MAR, UPW areas) to 30 m (GYR area). **(a)** Box-plot distribution of the factor of enrichment in the non enriched control after 24 h incubation under in situ-simulated conditions. **(b)** Box-plot distribution of enrichment factors obtained after 24 h incubation in presence of nitrate+nitrite (N), glucose (G) and phosphates+nitrates+nitrites+glucose (NPG). Enrichment factor is the leucine incorporation rate after 24 h amendments compared to the leucine incorporation rate in the non-amended control at the same time. MAR – St 5 group (site MAR and stations STB1 to STB5, n=6), St 6-St 15 group (stations STB6 to STB8, site GYR, stations STB11 to STB15, n=9), EGY-UPW (site EGY, stations STB17 to STA21, UPW1, UPW2, n=8). The middle line in the box is the median value. Horizontal lines (set to 2) refer to the level below which the effect of enrichment is considered insignificant.

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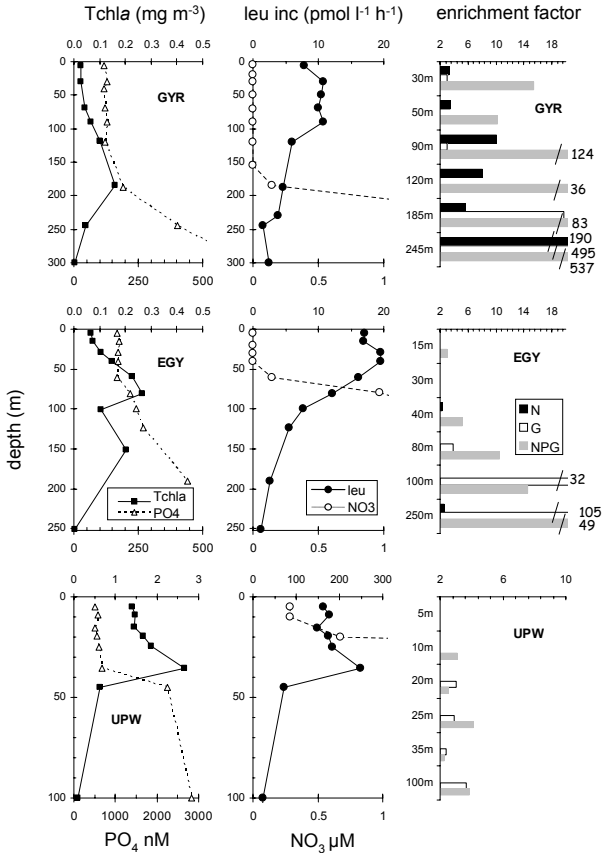

Fig. 5. Distribution of in situ chlorophyll a, leucine incorporation rates, nitrates and phosphates concentrations along vertical profiles, at sites GYR, EGY and UPW and responses to bioassays. Bioassays are expressed in terms of stimulation factor after N, G and NPG additions (leucine activity divided by leucine activity in the control at the same time). Only stimulation factors greater than 2 were plotted.